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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Atty. Docket: EINAT4.1C

In re Application of:	)	Conf. No.: 7736
Paz EINAT et al	)	Art Unit: 1637
Appln. No.: 09/802,472	)	Examiner: K. Young
Filed: March 9, 2001	)	Washington, D.C.
For: SEQUENCES CHARACTERISTIC OF HYPOXIA-REGULATED GENE TRANSCRIPTION	)	

DECLARATION UNDER 37 C.F.R. §1.132

Honorable Commissioner for Patents
U.S. Patent and Trademark Office
2011 South Clark Place
Customer Window, Mail Stop Amendments
Crystal Plaza Two, Lobby, Room 1B03
Arlington, VA 22202

Sir:

I, the undersigned Dr. Peter Chumakov, hereby declare and state as follows.

1. I have worked closely with the inventors of the above-identified patent application on experimentation relating to said invention.

2. In the above-identified application, the following statements were made by the inventors:

(a) Page 38, line 21, to page 39, line 6:

> Bad genes are useful in that they can be used in diagnostic assays for cells that have been subjected to hypoxia or ischemia. If mRNA corresponding to such genes, or the translation product thereof, is found in the cells being assayed it is likely that they have been subjected to hypoxia or ischemia. If diagnosed pre-stroke, this may be predictive of incipient stroke. They are also useful as a target for assays for the discovery of drugs which selectively down-regulate such genes or are otherwise dominant negative with respect to the

expression of the gene product of such genes.

(b) Page 40, line 17, to page 41, line 8:

As all of the genes of the present invention have been found to be modulated significantly upward after the cells have been subject to hypoxia, all of such genes may be considered to be a gene of interest for the purpose of the diagnostic assays reported herein.

Methods of detecting tissue hypoxia in mammalian tissue are based on the use of the mRNA of the genes of interest or the translation product thereof as a diagnostic marker for cells that have been subjected to hypoxia or ischemia. It is possible to determine the level of the mRNAs or protein translation products corresponding to these bad genes, in normal tissue or bodily fluids as compared to hypoxic tissue a bodily fluid from a subject which has suffered a hypoxic event, and, thus, determine the reference values of these genes on mRNAs or proteins which are indicative of tissue hypoxia.

3. Under my direction and control, a series of experiments has been performed as follows:

(a) Polyclonal antibodies against gene 95 (also referred to herein as "Hi95" for "Hypoxia induced (gene) 95") polypeptide were generated and tested for specificity (details are presented in the Appendix, Experiment A).

(b) An experiment was designed and conducted in order to verify the induction of the expression of the gene 95 polypeptide as a result of oxidative stress events (details are presented in the Appendix, Experiment B).

(c) An experiment was designed and conducted in order to verify the induction of the expression of the gene 95 polypeptide as a result of DNA damage (details are presented in the Appendix, Experiment C).

4. From the above experiments, the following conclusions can be drawn:

(a) Affinity purified antibodies against the gene 95 polypeptide recognize both the exogenous (Figure 1) and the endogenous gene 95 human polypeptide (Figures 2 and 3) with high specificity.

(b) Elevated expression of the gene 95 polypeptide following treatment of cells with H_2O_2 was detected in all cell lines tested (Figure 2).

(c) Induction of gene 95 polypeptide expression was completely abolished in RKO cells in which p53 expression was inactivated via RNA interference. In contrast, both types of cells retained the ability to strongly induce the gene 95 polypeptide in response to H_2O_2 (Figure 3).

(d) Thus, in addition to elevation of gene 95 mRNA levels, the expression of the gene 95 polypeptide is indeed induced in humans in response to oxidative stress.

5. Thus, these experiments provide further experimental evidence for the statements described in item 2 above, i.e., that the gene 95 polypeptide is useful for diagnostic assays.

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

January 10th 2005
Date

Peter Chumakov
Peter Chumakov